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TECHNICAL STUDY 33

RIFT VALLEY FEVER
A REVIEW OF THE LITERATURE

JULY 1961

U.S. ARMY CHEMICAL CORPS
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U.S. ARMY CHEMICAL CORPS RESEARCH AND DEVELOPMENT COMMAND
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Technical Study 33

RIFT VALLEY FEVER

A Review of the Literature

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FOREWORD

The literature pertaining to Rift Valley fever has been reviewed in conjunction with investigation of the disease and its etiologic virus. The work was performed under Project 4B11-02-065.

Intended as an economic measure, it has been addressed to the consolidation and summarization of published material of potential value in the study, research, and evaluation of Rift Valley fever.

The number of reports pertaining to this disease and its agent was limited by the number of laboratories engaged in investigations of the disease since its discovery in 1931. Approximately 180 reports were reviewed. The number of reports published world-wide was estimated at about 200. Because of duplication only about 100 of the available reports were cited in this text.

DIGEST

Rift Valley fever literature was reviewed with the intention of accumulating, under a single cover, published material of use in the study, research, and evaluation of the disease. Emphasis was placed on presentation of facts as they were reported in scientific publications.

Attention has been given to (a) geographic distribution, (b) modes of transmission, (c) susceptibility of hosts, (d) pathology, (e) immunity, (f) characteristics of the virus, and (g) investigation procedures.

Rift Valley fever has been shown to be a highly infectious disease of sheep, cattle, and other animals. Man has been infected frequently during epizootics of domestic animals and during laboratory or field contact with infectious material. The disease has not been found to occur naturally outside the African continent, but accidental human infections have been reported in the United States, Europe, and Japan.

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I. INTRODUCTION

A. DEFINITION

Rift Valley fever has been defined as an acute, febrile, insect-borne virus disease of sheep, cattle, and other animals. Also susceptible, man has been infected during epizootics of domestic animals and laboratory accidents.

B. HISTORY

In 1930, Daubney et al.[1/*] isolated a specific virus of the disease during an epizootic in sheep and cattle of the Rift Valley in Africa. The Rift Valley was described as a huge geological depression that starts in Persia and continues through northeastern and central Africa until it ends in eastern Transvaal.[2/] However, the disease was not confined to the Rift Valley, and it probably existed in Africa much earlier than 1931. According to Weiss,[3/] Montgomery (1912) and Stordy (1913) reported the existence of a disease in man and domestic animals with a symptomatic resemblance to Rift Valley fever.

Daubney first designated the disease "enzootic hepatitis" because of extensive liver damage in infected animals. Because pathology extended to other organs as well, he later decided upon the name Rift Valley fever, the designation consistently employed in the literature.[1/]

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* See Literature Cited.

II. RIFT VALLEY FEVER

A. GEOGRAPHIC DISTRIBUTION

Evidence of Rift Valley fever has been found in a wide belt extending from the southern tip of the Union of South Africa northward into Central Africa.[4] Occurrences of the disease in this belt and elsewhere are shown in Figure 1.

The first proved epizootic, described by Daubney in 1931, occurred in Kenya.[1] The disease was contracted by shepherds attending infected flocks and by four Europeans engaged in examination of infected animals and in laboratory work with infected materials.

Transmission of the virus by mosquitoes and presence of the disease in Uganda was reported by Smithburn et al. in 1948, when virus was isolated from mosquitoes caught in the uninhabited Semliki forest.[5] Even before this, Findlay et al.[6] had shown that virus-neutralizing antibodies existed in the sera of natives in Uganda, French Sudan, Anglo-Egyptian Sudan, and French Equatorial Africa. Another outbreak of the disease occurred in Kenya in 1933.[7] In the summers of 1950 and 1951 the first epizootic occurred in the Union of South Africa.[8,9] The South Africa epizootic involved the western and southwestern areas of Orange Free State, northern and western areas of Cape Province, and the western and southern areas of Transvaal.[10] Epizootics reappeared in the Union of South Africa in 1953 (Fauresmith district), 1955 (Marienthal district), and in the summer of 1956 (Western Orange Free State).[3,11-13] In 1957 Kokernot et al.[14] found that wild-caught mosquitoes in Zululand carried the virus and Shone reported the presence of antibodies in the sera of cattle in Southern Rhodesia.[15]

Kaschula[17] found antibodies in the sera of cattle in the Knysna district, but no evidence of clinical disease other than an occasional abortion in cattle. He suggested that these areas formed truly enzootic areas similar to the Semliki forest. Enzootic areas were described as low, warm areas with high annual rainfall. Similar terrain and climate plus the added factor of sheep farming characterized the epizootic areas. It was reasoned that the disease reached epizootic proportions only in those enzootic areas that were also suitable for sheep, a highly susceptible host.[3,18] The disease halted abruptly with the first frost and with movement of herds to high altitudes.[1,7] The geographic distribution of arthropod-borne viruses in South Africa was investigated in some detail by Kokernot et al.[14,18-20] and Paterson et al.[21]

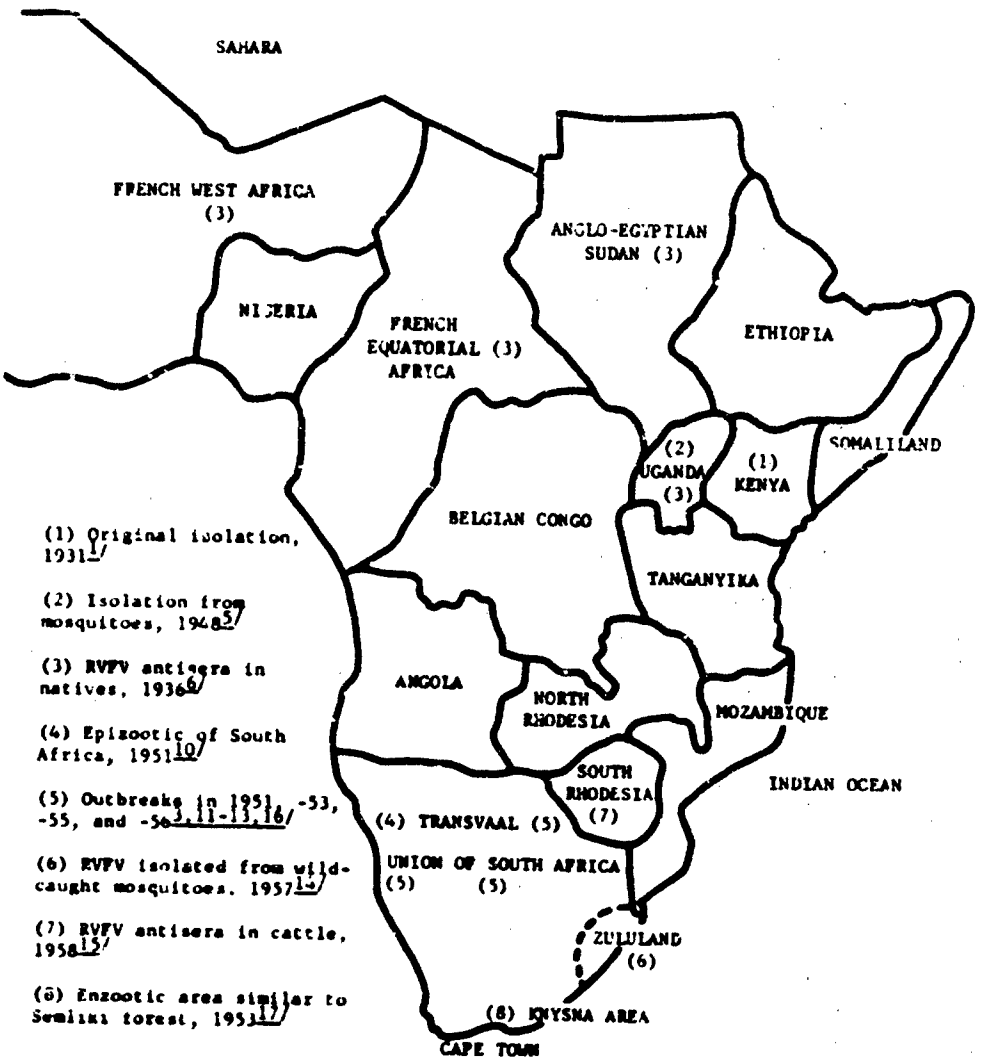


Figure 1. Geographic Distribution of Rift Valley Fever in Africa.

B. SUSCEPTIBILITY

A wide range of species was classified according to their susceptibility to laboratory infection with Rift Valley fever by Findlay et al.[22-24] He classified lambs, mice, hamsters, and wild rodents as most susceptible, inasmuch as the disease was usually fatal to these animals. It was also fatal to about fifty per cent of the second group, composed of sheep and rats. Man, monkeys, cows, goats, and grey squirrels experienced severe nonfatal infections and were placed in a third group. Cats were next with a mild reaction, followed by rabbits, which showed no evidence of infection although the virus persisted in the blood for several days. In a study of susceptibility among various species of monkeys, Findlay reported differences in susceptibility that were separate from differences in antibody response.[25] Indian, African, and South American monkeys were equally susceptible, although certain species of African monkeys were less susceptible. The more susceptible group exhibited a febrile reaction and viremia in contrast to certain African monkeys, which showed nothing more than the circulation of virus for a few days. In 1952 Findlay encountered differences in the susceptibility of mice and rats in relation to age and diet.[26] Suckling mice were more susceptible than older mice and rats. Rats maintained on an inadequate diet were more susceptible than well-fed rats. Findlay pointed out that the diet upon which the rats were maintained for the susceptibility studies in 1932 was not considered adequate in the 1952 studies. High death rates indicated that ferrets should be placed in the group of highly susceptible animals.[27] Horses, pigs, monkeys, hedgehogs, porcupines, frogs, and domesticated and wild fowl were listed as nonsusceptible by Findlay.[22]

C. TRANSMISSION

1. Natural

Seasonal aspects of Rift Valley fever outbreaks coincident with prevalence in low terrain following periods of heavy rainfall led Daubney et al. to conclude that the virus was transmitted naturally by biting insects.[1] Furthermore, they were able to stop the disease by moving flocks to higher altitudes or sheltering animals from mosquitoes. The mosquito was established as a vector by Smithburn et al. in 1948 when the virus was isolated from mosquitoes caught in the Semliki forest of Uganda.[5] Virus was isolated from three species of the genus Aedes and six species of the genus Eretmapodites. Material from the Aedes species had lower titer than that from the Eretmapodites species. It was thus concluded that an Eretmapodites species was the vector and the Aedes species were incidentally infected and not involved in the parasitic cycle. In 1949 the virus was transmitted from lamb to lamb and from mouse to mouse in the laboratory with Eretmapodites chrysogaster.[28] Epidemiological studies in 1948, 1951, and 1953 showed no evidence of person-to-person spread or infection of man by an insect vector.[5,12,29] Virus isolations from wild-caught mosquitoes are summarized in Table I.

TABLE I. RIFT VALLEY FEVER ISOLATIONS FROM WILD-CAUGHT MOSQUITOES

SPECIES	LOCATION	DATE	LITERATURE CITED
Aedes deboeri de-meilloni	Africa	1948	5
Aedes tarsalis	Africa	1948	5
Aedes caballus	South Africa	1955	13
Aedes circumluteolus	South Africa	1956 & 1957	20,30
Aedes africanus	South Africa	1956 & 1958	30
Eretmapodites chrysogaster	Africa	1948	5
Culex theileri	South Africa	1955	13

2. Experimental

Rift Valley fever virus has been transmitted in the laboratory by scarification, injection, nasal instillation, eye instillation, injection by biting insects, and sometimes by eating infected material. Vectors that have been induced to transmit the virus in the laboratory and those that have become infected without transmitting the virus are shown in Tables II and III, respectively.

TABLE II. VECTORS WITH WHICH EXPERIMENTAL TRANSMISSION OF RIFT VALLEY FEVER VIRUS HAS BEEN ACHIEVED

SPECIES	DATE	LITERATURE CITED	REMARKS
Rhipicephalus appendiculatus (tick)	1933	7	Virus not retained thru molt to adult
Eretmapodites chrysogaster	1949	28	
Aedes aegypti	1955	31,32	Adapted viscerotropic Lunyo strain only
Aedes caballus	1955	13	
Aedes tarsalis	1954	2	
Mansonia species	1954	2	

TABLE III. MOSQUITOES THAT WERE INFECTED WITH RIFT VALLEY FEVER UNDER LABORATORY CONDITIONS BUT DID NOT TRANSMIT VIRUS

SPECIES	DATE	LITERATURE CITED
Culex theileri	1955	33
Mansonia fuscopennata	1933	7
Mansonia versicolor	1933	7
Mansonia microannulata	1933	7

The immediate portal through which the virus infects during ingestion of food has not been clearly shown. Daubney et al. failed to transmit the virus by drenching a lamb with infected blood.[1] Yet mice have been infected by allowing them to feed on other mice that were moribund or dead of Rift Valley fever.[32] It was pointed out that actual transmission could have occurred in several ways. Experimental transmission of virus by nasal and eye instillation[34] and scarification suggested that infection could have resulted from cage-dust aerosol, aerosol created by clawing at infected mice, or scratches incurred during eating, but probably not from simple ingestion of infected material.[22] Experimental transmission of the virus to a human volunteer is described in the section on human infections.

3. Accidental

Exact portals of entry responsible for transmission of the virus in accidental infection of veterinarians and laboratory workers remain somewhat obscure. The first reported infections followed participation in post-mortem examination of infected animals and laboratory exposure in which little or no protective paraphernalia was used.[1] Later, varying degrees of protection including gloves, surgical masks, and protective over-garments were used without success. Accidental infection has also resulted from handling contaminated glassware,[32] entering rooms housing infected animals, pouring virus suspensions, and grinding infected tissue in mortar and pestle.[33] One accidental infection occurred under circumstances that suggested unusual resistance of the virus to drying.[27] An individual, whose presumed contact with the virus was scraping and painting the walls of a room that had housed infected laboratory animals three months earlier, became infected 15 days after the paint-scraping experience. Active work in that location with Rift Valley fever virus had been terminated four months before the onset of illness.

Findlay believed that the virus could gain entry through skin abrasions, the conjunctival sac, or the mucous membrane of the nose.[22]

Investigators have failed to isolate the virus from the urine of infected man or animals, but it has been isolated from muco-hemorrhagic feces[36] and material aborted by infected ewes.[17]

Search has been made for an intermediate host that circulated an adequate titer of virus long enough to participate in the parasitic cycle. Weinbren and Mason[37] observed that a wild field rat, Arvicanthis abyssinicus, had antibodies to Rift Valley fever in its serum and investigated the animal as a possible intermediate host. They concluded that the rodent could act as a natural host to the virus.

D. HUMAN INFECTIONS

1. Sources

Rift Valley fever infections acquired under natural conditions occurred in humans during each recorded epizootic of sheep and cattle.[3] Infections occurred among personnel engaged in post-mortem examination of diseased animals, among laboratory personnel in contact with infectious materials and among others whose occupations involved them with infected animals.[2]

2. Experimental Infection

Daubney et al.[1] provided the only report of a human experimental infection with Rift Valley fever virus. No apparent risk was involved, because earlier accidental infections were without known fatalities. An adult male of the Kissi tribe, a malarial patient, was given three cubic centimeters of diluted virus intramuscularly. The inoculum was prepared from 0.2 cc of lamb plasma filtrate which was obtained with a Chamberland L5 filter. Titration of the material was not reported, but the patient probably received 10^7 to 10^8 MLPLD$_{50}$ of virus.

On the third day after inoculation, the patient complained of headache and pain in the loins. On the fourth day, symptoms became severe and the febrile response appeared. The face was congested, eyes were slightly bloodshot, and the pulse was rapid. On the fifth day the temperature returned to normal but abdominal discomfort remained for weeks. Viremia was detected from the fourth through the ninth days after inoculation. No untoward sequelae were reported.

3. Confirmed Infections

Rift Valley fever infections in humans have been heavily documented from case reports of persons contracting the disease during occupational contacts with the virus. Reports of confirmed cases have been summarized in Table IV. Note that the first such report was made by Daubney and co-workers in 1931. Infections were contracted in every laboratory in which work with Rift Valley fever was carried on. Infections of African, European, Japanese, and American workers in a variety of occupations were reported. Most infections followed known contact, but contact was not always well defined. High morbidity among those exposed to the virus was apparent from the earliest reports.

4. Morbidity and Mortality

Daubney et al.[1] and Findlay[22] reported 100 per cent morbidity among personnel following post-mortem experience with infected carcasses. Almost every native engaged in herding sheep during the 1930 epizootic contracted a disease with symptomatic resemblance to Rift Valley fever. Subsequent to the 1950 outbreak in South Africa, Schulz estimated 70 per cent morbidity among approximately 32,000 people assumed to be directly exposed.[10] Ten to fifteen per cent morbidity was estimated for the total population assumed to be at risk, approximately 500,000 people. The number of persons physically involved with infectious materials was much smaller. All state veterinarians, stock inspectors, and persons known to take an active part in post-mortem examination of diseased animals contracted the fever. Thus, the morbidity among susceptibles positively exposed was 100 per cent; lower occurrence in the general population probably reflected degree of exposure rather than morbidity.

In contrast to high morbidity, only one human fatality associated with Rift Valley fever has been reported.[39] This report is reviewed in Section II, D,5,d.

5. Clinical Picture

a. Symptoms

Remarkably similar symptomatically to dengue fever, Rift Valley fever has a sudden onset with elevated temperature, headache, muscular pain, weakness, sensation of fullness over the liver, rigors, vertigo, photophobia, nausea, and sometimes constipation and epistaxis.[3] Elevated temperature frequently occurs in two phases.[1,22]

b. Incubation Period

After three to six days' incubation period, a rise in temperature occurs, along with one or more of the symptoms previously described,

TABLE IV. SUMMARY OF RIFT VALLEY FEVER INFECTIONS IN HUMANS

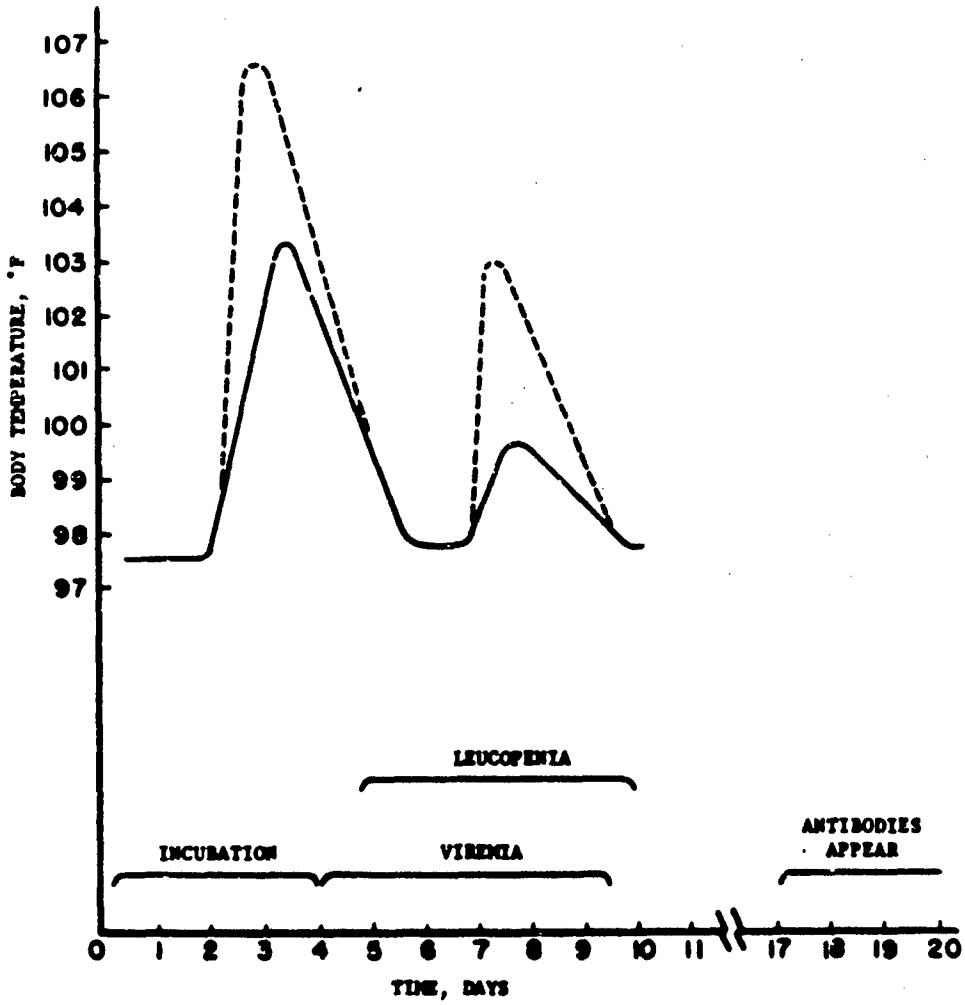
PLACE	NO. OF CASES AND OCCUPATION	CONFIRMATION TEST[a]	LITERATURE CITED
Kenya	2 veterinary surgeons 2 lab assistants	None	1
London	1 pathologist 1 veterinary surgeon 3 lab assistants	SN SN V,SN	22,38
United States	1 pathologist 3 technicians 2 lab assistants 2 virologists	V,SN	27,35,38,39
Uganda	2 pathologists 6 technicians 3 animal caretakers	V,SN	40
Japan	12 lab workers	CF,SN	41
South Africa	14 farmers 7 veterinary surgeons 2 teachers 2 research station employees 1 merchant 1 diamond worker 1 technician 2 natives 8 unknown	V,SN,CF	12,15,17,42-47

a. SN = serum neutralization
V = virus isolated
CF = complement fixation

and persists for two to three days (Figure 2). The temperature frequently returns to normal for one or two days and rises a second time. Temperatures reach 103° to 107°F, usually accompanied by symptoms.[3]

c. Incapacitation

The febrile response more or less paralleled the period during which virus was isolated from the blood, a period of four to six days.[22]



Adapted from Daubney et al.[1] and Findlay[22]

Figure 2. Clinical Signs of Rift Valley Fever Infection in Humans.

This period was also marked by a sharp rise in the leucocyte count, which changed to leucopenia as the febrile period terminated. Incapacitation varied from inapparent infections to complete debilitation. However, clinically recognizable cases usually required bed rest during the febrile period. Recovery was most often uninterrupted and complete, but malaise, weakness, and complaints of headache and defective vision were reported to persist for several weeks.[3]

d. Sequelae

(1) Ocular

Symptomatic visual disturbances were noted frequently in reports on human cases of Rift Valley fever. Photophobia, tenderness of the eyeballs, and pain behind the eyes were frequently reported.[44]

Daubney et al.[1] reported that a laboratory worker complained of defective vision for some weeks following the disease. The nature of the visual defect was not described, nor was there any reference to investigation of the complaint. Although complaints of visual disturbance have been made following Rift Valley fever infections in Central Africa, evidence of retinal damage following infection has been reported only in the Union of South Africa.[32]

Central serous retinopathy, characterized by macular swelling and occasional small hemorrhages, was described by Freed[44] and Schrire.[45] Freed reported the case of a schoolmaster, 38 years old. who complained of visual disturbance of six weeks' duration in the left eye. Six days after onset of illness the patient noticed blurring of vision. Six weeks later examination showed the cause to be a dense white elliptical mass covering the left macula. Previous exposure to Rift Valley fever virus was shown by the complement-fixation test.

Schrire[45] described five cases of macular exudates and one case of retinal detachment seen in his practice during the 1951 epizootic in the Union of South Africa. All of these cases were diagnosed by the complement-fixation test. Data pertinent to these cases are summarized in Table V. The onset of eye symptoms varied from the beginning of the febrile period to three weeks later. Visual defects remained in most of the cases for more than two months. In one case they were permanent. In some, after many months, the lesions resolved; in others, complications persisted for three years.[13] During the South African epidemic of 1951 several ophthalmologists noted similar changes, but these have not been reported in detail.[45]

The relationship between Rift Valley fever and visual sequelae has not been definitely established. Most of the observations reported were on patients without continuous history. Diagnosis of

TABLE V. OCULAR SEQUELAE IN HUMAN CASES OF RIFT VALLEY FEVER

CASE[a] NUMBER	AGE	OCCUPATION	CONTACT WITH ANIMALS	ONSET OF EYE SYMPTOMS AFTER PYREXIAL ATTACK	FUNDAL FINDINGS	PERSISTENCE OF VISUAL DEFECTS	CF TEST
1	34	Teacher	No	1 week	Large macular mass & small hemorrhage in left eye	>2 months	+
2	34	Research station worker	Yes	1 week	Large macular mass in left eye	>2 months	+
3	29	Research station worker	Yes	3 weeks	Very small macular exudate in left eye	>3 months	+
4	28	Merchant	No	2 weeks	Large paramacular mass in left eye	>1 month	+
5	40	Diamond classifier	No	2-3 days	Large paramacular mass in right eye	Between 6 and 12 weeks	+
6	50	Veterinary surgeon	Yes	Same time	Retinal detachment in right eye	Permanent	+

a. All cases were in males.
Modified from Schrire.[45]

Rift Valley fever was made serologically, i. most instances weeks or months after the discovery of past illness related by the patient. In three of the six cases reported by Schrire there was, however, a history of contact with animals. Two patients (Cases 2 and 3) were employed at a government research station where a number of cattle were found to have Rift Valley fever. At the same time five other staff members had an influenza-like disease. The third case (Case 6) was a government veterinarian who investigated an outbreak in cattle. This evidence, coupled with the complement-fixation tests, circumstantially linked Rift Valley fever with the reported visual disturbances.

(2) Circulatory

Continuous history of the only reported fatality associated with Rift Valley fever was also inconclusive. Schwentker and Rivers[39] reported the history of a pathologist, age 30 years, who contracted the disease in the laboratory. The patient exhibited a typical clinical case of infection and recovery until the sixteenth day after onset, when phlebitis developed in the popliteal vein of the left leg. On days 20 and 26 pulmonary infarcts formed in the right lung. Another infarct formed in the left lung on day 34 and phlebitis developed in the femoral vein of the right leg four days later. The patient died from a large pulmonary embolus on day 45. Autopsy revealed none of the pathological changes associated with Rift Valley fever infections of larger animals. Typically, virus was isolated from the blood on days 1 through 3, but not thereafter. Joubert et al.[48] referred to one individual who developed coronary thrombosis one week after contracting Rift Valley fever, but stated that the patient was of an age and weight frequently associated with circulatory disorders.

6. Immunity

a. Duration

Antibodies appeared in the sera of infected persons 14 days after onset[49] but the duration of active immunity in man following Rift Valley fever infection has not been established.[50] Findlay[51] reported antibodies in the sera of laboratory workers who had recovered from infection four to five years earlier, but they had had subsequent contact with virus. Sabin and Blumberg[33] reported antibodies in the serum of a patient infected and without known contact with the virus 12 years after recovery. Findlay and Howard[52] reported on the duration of immunity in the laboratory workers first reported in 1936. The sera of all the workers retained antibodies, which had persisted in the absence of exposure to the virus for periods of 12, 18, and 20 years. Schrire and Gear[43] claimed that antibodies persisted for 20 years following infection, and suggested that recovery might be followed by life-long immunity. Brown et al.[50] questioned the 20-year figure because protocols were not reported, but presented evidence of antibodies persisting for 25 years. Neutralizing

antibodies were demonstrated in the serum of one of the authors (T. Dalling) 25 years after recovery from infection. Expressed as the reciprocal of the 50 per cent neutralizing dilution of 250 mouse subcutaneous doses, the serum titer was $1 \times 10^{1.8}$. Dalling had no further contact with the virus after the infection.

b. Immunization

(1) Passive

Findlay[22] apparently avoided clinical infection by administering immune human serum during the period of exposure to the virus. He reported the appearance of previously undetected antibodies in his serum without experiencing apparent infection.

(2) Active

Randall[53] developed a vaccine for Rift Valley fever, using formalin-inactivated virus which propagated in monkey kidney-cell tissue culture. The vaccine was tested in animals and human volunteers. Neutralizing antibody titers in animals and volunteers were comparable. Antibody titer and immunity were correlated by intracerebral challenge in mice.

Vaccine produced from monkey kidney-cell tissue culture was prepared by inoculating cells in serum-free medium 199 with virus and incubating for 96 to 144 hours at 36°C. Infected whole cultures were homogenized in a Waring blendor and clarified by centrifugation. The supernatant blend was passed through a sintered glass filter of medium porosity and the pH was adjusted to 7.0 to 7.2. If the material was of high titer ($10^{8.3}$ to $10^{8.9}$ per ml), it was treated with formalin in a final concentration of 1:1000. Free formalin was neutralized with sodium bisulfite and the vaccine was then dialyzed against Hank's balanced salt solution for 24 hours at 4°C. The vaccine was tested for bacterial sterility and viable virus at several time intervals and then safety-tested in the final procedure.

E. ANIMAL INFECTIONS

1. Natural Infections

Alexander[8] and Dickson[9] classified Rift Valley fever in sheep and cattle according to clinical signs of the disease. They recognized a peracute form, an acute form, a sub-acute form, and a mild or inapparent form. The peracute form was common in very young lambs. An incubation period of about 12 hours was followed by collapse and death within 36 hours in 95 to 100 per cent of the infected lambs. During the 24 hours preceding death, lambs were listless, disinclined to feed, and sank down soon after being put on their feet. The absence of diarrhea was noted. The acute form was

commonly encountered in lambs and to a lesser extent in adult sheep. Clinical signs appeared suddenly and included a rapid rise in temperature, vomiting, mucopurulent discharge from the nose, rapid pulse, unsteady gait, and abortion in pregnant animals. Death usually followed onset within 24 to 48 hours. The mortality rate was high in lambs and varied from 20 to 30 per cent in adult sheep. In adult sheep and cattle, the subacute form was common. Body temperatures rose to 104° to 106°F and persisted for 24 to 96 hours. There was inappetence and general weakness. Abortion was frequently the only sign in pregnant animals. Milk production decreased rapidly. The mortality rate was low, less than 10 per cent in cattle. The mild or inapparent form also occurred in adult sheep and cattle. The only sign of disease was a mild febrile reaction, and diagnosis could be made only by serological methods. Leucopania followed the apparent forms of infection.[22]

2. Experimental Infections

Experimental infection of lambs with pantropic Rift Valley fever virus (PRVFV) was followed by a clinical picture similar to natural infections.[1,22] Goats showed signs similar to those of sheep. Cattle showed dullness, inappetence and blood-stained nasal discharge.[55] Sheep and lambs inoculated with neurotropic Rift Valley fever virus (NRVFV), other than intracerebrally (IC), exhibited a mild or inapparent form of the disease.[17,56] However, when inoculated IC, NRVFV caused a rapidly fatal encephalitis. Death occurred within 24 hours without signs other than a slightly elevated temperature, but if the animal survived 48 to 72 hours, the elevated temperature was followed by retraction of the head, inability to rise, and convulsive twitching of the limbs.

Mice and rats experimentally infected with NRVFV and PRVFV exhibited roughened coats, lethargy, tremors, convulsions, subnormal temperatures in later stages, coma, and usually death in one to three hours after onset of disease and within 36 to 72 hours after inoculation.[36,57] Pregnant mice and rats frequently aborted and died. The young were still-born or died shortly after birth.[17]

A febrile, nonfatal form of RVF developed in monkeys within 24 to 96 hours after inoculation.[25] The febrile response persisted for 24 to 120 hours, followed by leucopenia, but other signs of disease were not apparent.

3. Pathology

Pathology of Rift Valley fever infection in various animals was investigated by Daubney et al.,[1] Findlay et al.,[22,52,56,58] Marschal,[59] Smithburn,[57] Kitchen,[34] Schulz,[60] and Mims[61] then reviewed by Weiss in 1957.[3] The material presented below was summarized from the observations published by these investigators.

a. Sheep, Cattle, and Goats

The pantropic virus primarily affected the liver, which showed characteristic focal necrosis.[3] Liver degeneration in lambs differed somewhat from that in sheep. In lambs, the liver was frequently yellow, rarely enlarged, but always lacking the deep red of the normal liver. It showed necrotic foci approximately one millimeter in diameter in association with subcapsular hemorrhages scattered beneath the capsule. The lesions extended throughout the liver and in peracute cases the normal architecture was sometimes completely lost. The parenchymatous cells underwent hyaline degeneration and lost their affinity for acidophilic stains. With the exception of a few cells near the central vein, the lobule was made up of irregular masses of lightly stained cytoplasm. Leucocytes and histiocytes showing karyorrhexis infiltrated between these masses.

The liver of mature sheep was usually mottled brown and frequently enlarged. Liver cells degenerated and the lesions accumulated polymorphonuclear leucocytes and histiocytes. Lesions were usually focal and not panlobular, as in lambs. Early changes of the liver were observed in a few cells in the central zone of the lobule. Hyaline bodies, indistinguishable from Councilman lesions of yellow fever, were formed by cloudy swelling of the cytoplasm, which was followed by hyaline degeneration. As the lesion progressed, the cells contracted and showed oxychromatic degeneration of the nucleus and development of inclusion bodies. Findlay[58] regarded the inclusion bodies as degeneration products. Progressively, the lesions were then invaded by polymorphonuclear leucocytes and histiocytes, which were destroyed and formed a necrotic mass. When the lesion was in the central zone this mass formed an occlusion of the central vein. Generally, liver lesions in cattle and goats were similar to those observed in older sheep.

The spleen in this group of animals usually showed subcapsular petechiae and capillary arborescence, primarily near the free borders. According to Schulz,[60] lesions observed during the Union of South Africa epizootic showed, as a rule, tumor splenis and subcapsular hemorrhages. Necrobiotic changes in the pulpa could be seen, along with occasional infiltration of neutrophiles.

The kidneys of lambs showed congestion of the cortical and medullary blood vessels, especially near the boundary zone. They showed cloudy swelling, and on occasion the cells of the convoluted tubules lost their ability to retain nuclear stains. Lesions in older sheep most often progressed to tubular degeneration or nephrosis.

The alimentary tract was inflamed in degrees varying from catarrhal to hemorrhagic enteritis. Schulz found large subperitoneal hemorrhages along the entire gastrointestinal tract. In addition to the usual enteritis, he also observed areas of croupous or necrotic enteritis and ulceration.

The wall of the gall bladder showed petechiae; it was often thickened from subserosal and muscular hemorrhage, hyperemia, and edema; it showed extensive desquamation and necrosis of the mucous membrane.

Cyanosis was observed in visible mucous membranes and skin, particularly in the udder, scrotum and axillary regions, the lower part of the extremities, and inside the hind legs. Subcutaneous tissues were edematous and the cutaneous blood vessels were distended. In addition, cattle showed acute catarrhal stomatitis, erosion of the lips, tongue, and cheeks; coronitis, laminitis, exungulation, and, on occasion, marked ascites.[60]

In the lungs, congestion of the meningeal vessels and interalveolar capillaries was noted. The lungs showed hyperemia, edema and emphysema and subpleural or perivascular hemorrhages. Schulz also observed signs of fibrinous pneumonia in lambs. Degenerative changes were observed in the adrenals. Cortical and medullary hemorrhages and a number of cells containing hyaline inclusions were observed.

The heart showed subpericardial hemorrhages in the region of the coronary grooves and small subendocardial extravasations in the left ventricle. The mesenteric and omental vessels were deeply engorged, and the mesenteric lymph glands were enlarged and moist. At times hemorrhages extended into the cortex of the glands.

The only abnormal change noted in the placenta was invasion of the uterine musculature and decidua by polymorphonuclear leucocytes, many of which were breaking down and undergoing karyorrhexis. Kaschula[17] isolated virus from a foetus aborted by an infected ewe and concluded that the virus could pass the placental tissues.

Neurotropic virus, inoculated intracerebrally, produced encephalitis in lambs.[56] Focal necrosis, degeneration of ganglion cells and nuclear inclusions in ganglion cells, perivascular infiltration, and infiltration of the meninges with leucocytes were observed.

Pantropic virus produced discrete necrotic foci irregularly distributed throughout the liver lobules of monkeys.[22] Foci varied in number, and hyaline degeneration of the cytoplasm was not as well marked as in sheep and goats. Neurotropic virus, inoculated intracerebrally, gave rise to encephalitis similar to that described in sheep.[56]

b. Mice

According to Weiss,[3] liver lesions were found to be essentially the same in all susceptible species of animals. Findlay,[22] Smithburn,[57] Kitchen,[34] and Mims[61,62] described pathology of the disease in mice. The appearance of the liver in infected mice resembled that of lambs; infected

rats showed less extensive damage.[26] Smithburn[57] and Kitchen[34] described encephalitic lesions in mice, similar to those described in lambs and monkeys, when neurotropic virus was inoculated intracerebrally into mice. Fixed neurotropic virus did not produce liver lesions in mice, but virus in its eighty-fifth mouse intracerebral passage in mice produced small foci of degeneration on the livers of mice inoculated intraperitoneally.

Mims[63] reported that mice ill with Rift Valley fever had little or no prothrombin in the plasma. Prothrombin levels of less than five per cent of normal and clotting times of 10 to more than 60 minutes were recorded. Mims attributed the hemorrhagic phenomena in Rift Valley fever infections in mice to this deficiency. He pointed out that the deficiency had also been reported in yellow fever infections of rhesus monkeys.

c. Miscellaneous Animals

Findlay[22] reported that post-mortem appearances in small rodents such as hamsters, dormice, wood mice, and field moles were very similar to those in lambs and sheep dying of infection with pantropic virus. Marschal[59] described histological changes in the livers of hamsters and mice infected with Rift Valley fever. His findings essentially confirmed the observations of Daubney[1] and Findlay.[22]

Cats showed very small foci of degeneration on the liver. Ferrets presented a pathological picture characterized by extensive development of edematous pulmonary consolidation with a scanty exudate of large mononuclear cells.[27]

4. Immunization

a. Passive

Administration of immune serum within 36 hours after exposure to Rift Valley fever virus was shown to protect newly born lambs.[64] Practically, this prophylactic method had many disadvantages, and a live or attenuated vaccine that would produce lasting immunity was needed.

b. Active

MacKenzie[65] prepared two vaccines, one by inactivation of virus with methylene blue in light and one by inactivation with formalin. A number of investigators studied the attenuation of virus undergoing serial intracerebral passage in mice.[3] Kaschula[17] found that live vaccine prepared from 86 mouse passages and ten egg passages caused abortion in pregnant animals. Live vaccine prepared from virus with 102 mouse passages and 54 egg passages appeared safe for use in cattle and sheep, although it was slightly less immunogenic than that with fewer passages. According to Weiss,[3] the latter vaccine has been used extensively in Africa with encouraging results.

F. DIAGNOSIS

Confirmed diagnosis of Rift Valley fever has been limited to laboratory methods such as isolation and identification of virus, serological tests, gross pathology, and histopathological studies, especially of the liver.[3] Reliance upon symptomatic criteria resulted in delayed recognition of the 1950-1951 outbreak in the Union of South Africa.[10] Rift Valley fever was initially confused with diseases such as enterotoxemia and bluetongue in animals and influenza in humans. Similarities to "three-day stiff-sickness" of animals and dengue, yellow fever, and sandfly fever in humans have also been described.[66]

G. TREATMENT

Specific treatment of Rift Valley fever has not been reported. Thus, in practice, treatment has been symptomatic.[3] Chemotherapeutic trials without success included cortisone and adrenocorticotropic hormone,[67] and prontosil, sulphanilimide, and allied drugs.[68] Partial activity against infections in mice treated with chloramphenicol[69] and some of the acridines[70] has been reported.

III. RIFT VALLEY FEVER VIRUS

A. RECOGNIZED STRAINS

1. Pantropic

RVFV isolated from its natural environment, i.e., an epizootic in African sheep or cattle populations, showed definite affinity for tissues derived from two and possibly three germ layers.[3] The virus produced extensive necrosis of liver parenchyma and in this sense was frequently referred to as viscerotropic and hepatotropic. Lesions in tissues of mesodermal origin were of questionable RVFV etiology; the term polytropic was proposed as a more accurate term than pantropic, which was originally applied to viral pathogenesis of all three germ layers.[34] The term "polytropic" has not been found in RVF literature other than in the publication in which it was proposed.

2. Neurotropic

A neurotropic variant (NRVFV) of PRVFV was obtained by IC passage in young mice.[34,57,71,72] A summary of changes in the virus during 90 passages through mouse brain is given below.

NUMBERED IC PASSAGES IN MICE	CHANGES IN VIRUS AFFINITY FOR MOUSE TISSUE
1-15	No apparent change.
15-20	Gained neurotropism and retained hepatotropism.
20	Hepatotropism lost but easily regained.
33-49	Neurotropism became more stable but could still be lost.
80-90	Neurotropic. Hepatotropism could not be completely regained.

Adapted from Kitchen.[34]

The sixty-eighth passage of virus isolated from a human on the first day of illness showed both tropisms; virus isolated on the fourth day exhibited only the hepatic attribute.[34] The circulation of eightieth-mouse-passage virus in rhesus monkeys suggested that low-passage neurotropic virus tended to revert to its original pantropic character when injected into monkeys.[3]

3. Lunyo Virus

In 1956 Weinbren et al.[32] isolated two strains of an agent similar to RVFV from mosquitoes caught in the Lunyo Forest on Entebbe peninsula. The virus produced hepatic lesions similar to those produced by PRVFV in mice, but the histopathological picture was different and there were serological and behavioral differences. A summary of similarities and differences between RVFV and Lunyo virus is presented below.

SIMILARITIES	DIFFERENCES
1. Lunyo antiserum neutralized RVFV.	1. RVFV antiserum did not neutralize Lunyo virus.
2. Lunyo virus with strong neurotropic properties readily yielded a viscerotropic strain with IP passage in mice.	2. Lunyo virus was transmitted by Aedes aegypti mosquito, RVFV was not.
3. Lunyo viscerotropic strain showed cross neutralization with PRVFV.	3. Mice infected with neurotropic Lunyo virus became hyperactive, ate any available material including their own appendages, and usually died in convulsions 5 to 12 days after inoculation. Mice infected with NRVFV exhibited inappetence, progressive loss of muscular control, and died within 2 to 3 days after inoculation.
4. Lunyo viscerotropic virus reverted to neurotropic when passaged through a mosquito. PRVFV reacted similarly when passaged through certain rodents.	4. Lunyo virus was less stable in storage (lyophilized in serum) than RVFV.
	5. Lunyo virus failed to yield a hemagglutinin. RVFV readily yielded hemagglutinin.

Adapted from Weinbren et al.[32]

B. MORPHOLOGY

PRVFV readily passed through Chamberland filters up to L11 grade but rarely passed through filter grade L13.[1] By filtration through gradocol membranes, particle size of PRVFV was estimated at 23 to 25 millimicrons.[49] Completely different estimates of RVFV particle size were obtained by ultracentrifugation.[73-75] PRVFV particle size was reported as 49.7 millimicrons and NRVFV (102 IC mouse passages, 30 egg passages, and 9 IC mouse passages) showed two particle sizes, 30.9 and 51.8 millimicrons.[73,75] NRVFV purified by ultracentrifugation remained viable longer than NRVFV in mouse brain preparations.[76]

C. PROPAGATION

1. Mice

Mice and rats of several varieties have been used to propagate PRVFV.[22] Mims[36] used White Swiss mice for propagation and quantitation of RVFV. The virus is 98 to 100 per cent lethal for mice in two to three days.[22,36] Mims showed that age of mice had little effect on peak titers in mice 2, 4, 6, 8, and 10 weeks old. The rate of blood titer increase was proportional to the quantity of virus in the inoculum, but the final titer was approximately the same. Peak titers were reached one to two hours before mice sickened and died. Mice inoculated intravenously (IV) or intracerebrally (IC) with 10^2 to 10^3 LD_{50} per ml* virus produced blood titers as high as 10^{10} LD_{50} per ml. Subcutaneous (SC) or intraperitoneal (IP) inoculation gave titers in blood approximately one log lower. Titers in brain and liver were usually one to two $\log_{10}$ lower than titers in blood.

2. Lambs

Daubney et al.[1] used the lamb for isolation and propagation of PRVFV. The virus infection was fatal in two to three days for 90 per cent of the three to seven-day-old lambs. The mortality rate decreased with age to approximately 20 per cent in mature sheep. Exact titers of PRVFV in lambs were not reported, but data presented by Smithburn[57] suggested that virus in the bloodstream was plentiful. Two-tenths ml of lamb blood drawn daily from the first through the third days of infection killed all of the mice inoculated intraperitoneally. The lambs were infected with approximately 1×10^4 mouse LD_{50} of virus given subcutaneously. Neurotropic RVFV was rarely fatal to lambs except when inoculated intracerebrally.[56] Smithburn was unable to demonstrate NRVFV in the blood of sheep or lambs during the first ten days following subcutaneous inoculation with approximately 1×10^4 mouse LD_{50} of NRVFV.[57] Availability of lambs was seasonal, however, and their continuous use for propagation of RVFV was impractical. The gestation period in sheep is approximately 150 days;[77] therefore, under natural conditions lambs are available only in the spring and late fall.

3. Embryonated Eggs

Saddington[78] propagated PRVFV on the chorioallantoic membrane of 9- to 10-day-old embryonated eggs. Embryos were harvested after five days and the membrane, liner, and amniotic fluid were titrated in mice. Titers were not reported, but it was stated that all three materials contained

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* LD_{50} in mice was calculated by the method of Reed and Muench.

virus; the liver was more consistently infectious for mice than membranes or amniotic fluid. Kaschula[17] propagated both pantropic and neurotropic strains of the virus in the yolk-sac and on the chorioallantoic membrane of embryonated eggs. Highest titers of virus ($1 \times 10^{5.5}$ mouse LD_{50}) were obtained at 34°C from 8-day-old eggs inoculated in yolk-sac with $1 \times 10^{2.0}$ mouse LD_{50} per 0.1 ml virus and harvested at death (approximately 48 hours).

4. Tissue Culture

MacKenzie[79] and Saddington[78] carried PRVFV through 12 subcultures in 9- to 10-day old chick embryo cells suspended in Tyrode's solution. Virus titers of approximately $1 \times 10^{4.5}$ mouse LD_{50} were obtained after incubation of virus and cell suspension at 37°C for four to five days.[79]

Endo[80] reported the development of a neurotropic variant by serial passage of PRVFV in a Maitland-type tissue culture of embryonic mouse brain.

In roller tube tissue culture, Takemori et al.[81-83] demonstrated the cytopathogenic effect of NRVFV and PRVFV on rat sarcoma cells, human embryo, and rat, mouse, and swine fibroblasts. Weiss[3] reported cytopathogenesis of NRVFV in lamb kidney cells grown in roller tube cultures. Weiss confirmed the finding of Takemori et al. that the cytopathogenic effect appeared within about two days and maximum virus titer (1×10^{6} to 1×10^{7} mouse LD_{50}) was reached prior to marked cell destruction, which was complete in four to six days.

PRVFV and NRVFV were titrated in tissue culture by plaque formation on rat sarcoma cells,[81] Chang's human liver cells,[84] and sheep kidney cells.[85] Randall[33] propagated PRVFV in monkey kidney cell tissue culture for production of vaccine.

5. Adsorption and Multiplication

a. In vivo

Mims[86] obtained data for PRVFV growth curves by titration in mice. He reported that most of the virus disappeared from the blood within the first hour. Between five and nine hours, depending upon inoculum size, there was an exponential rise in titer. Comparable final yields of virus (1×10^{8} $MICLD_{50}$ per 0.03 ml) were obtained for different-sized inocula at different times, as shown below.

INOCULUM, $MICLD_{50}$ PER 0.03 ml	APPROXIMATE TIME, HOURS
$1 \times 10^{0.7}$	50
$1 \times 10^{1.5}$	48
$1 \times 10^{4.5}$	30
$1 \times 10^{7.3}$	18

Adapted from Mims.[86]

Two-step growth curves developed when inocula of 1×10^6 to $1 \times 10^{7.5}$ $MICLD_{50}$ per 0.03 ml were used. The curve was characterized by an exponential rise at four to five hours, when a one-hour lag appeared that was followed by a second exponential rise. One-step growth curves resulted from increasing the inoculum size to $1 \times 10^{8.3}$ to $1 \times 10^{8.5}$ $MICLD_{50}$ per 0.03 ml. Titers increased exponentially between four or five hours and seven or eight hours without evidence of lag. Peak titers of $1 \times 10^{8.5}$ to $1 \times 10^{9.0}$ $MICLD_{50}$ were reached in seven or eight hours.

Takemori et al.[82] constructed growth curves for both neurotropic and pantropic RVFV from data obtained by titration of ascitic fluid from infected mice that had been inoculated with ascites hepatoma cells eight days before virus inoculation. Matumoto et al.[87] confirmed the findings with neurotropic RVFV.

b. In vitro

Plowright and Ferris[85] reported growth curve studies of RVFV using sheep kidney monolayers in culture tubes. They reported peak titers of $1 \times 10^{6-6.5}$ $MIPLD_{50}$ in about 36 hours. Yakemori et al.,[83] using ascites hepatoma cells of mice in roller tube tissue culture, made quantitative measurements of NRVFV and PRVFV. They found little difference in the in vitro growth of the two virus strains. Peak titers of 1×10^6 $MICLD_{50}$ were attained after two to three days.

Iwasa[84] employed plaque formation on monolayers of Chang's human liver cells (CHL) to investigate the latent period and the appearance of intracellular antigen of RVFV. He stated that the period of intracellular multiplication was 2.5 hours and the latent period extended to 6 hours. Using the indirect fluorescent antibody technique, he detected intracellular complement-fixing antigen at 5 hours.

c. Interference

Findlay and Howard[52] investigated interference between neurotropic and hepatotropic virus and between ether-inactivated neurotropic virus and hepatotropic virus. Exaltation between Senger virus and neurotropic virus was also studied. They claimed correlation between the development of neurotropism and increasing interference between neurotropic and hepatotropic strains. Because of difficulties encountered in obtaining completely inactivated virus by ether treatment, these results were not clear-cut, but the authors concluded that ether-inactivated virus interfered with living virus. It was also concluded that Senger virus, a member of the encephalomyocarditis group of viruses, was exalted by neurotropic virus injected intraperitoneally.

Naude and Polson[88] investigated interference between active RVFV and ultraviolet-irradiated RVFV. They found that irradiated virus interfered with infectivity and that optimal doses of irradiated virus elicited immunity in mice. Joya[89] investigated the antigenic power of RVFV inactivated by UV irradiation.

Scott and Whitcomb[90] investigated interference of RVFV by rinderpest virus (RV) in hamsters and mice. Hamsters that were infected with RV two to seven days earlier were not affected by exposure to RVFV. The survivors were found to be susceptible to RVFV when re-exposed. Exposure to RV 8, 9, and 10 days before exposure to RVFV resulted in 50 per cent deaths from delayed RVFV infection. Survivors were immune upon re-exposure to RVFV. Periods of 1, 12, 13, and 14 days pre-exposure of hamsters to RV produced no evidence of interference on inoculation with RVFV. Similar evidence of interference did not occur in mice. The only significant feature of the mouse experiments was a delay in peak death time. Similarity of Rift Valley fever to dengue and yellow fever induced Findlay[22] to investigate interference among these viruses. He reported no interference between RVFV and dengue virus. However, Findlay and MacCallum[91] reported that IP inoculation of mice with a mixture of PRVFV and neurotropic yellow fever virus (NYFV) completely protected a few mice and delayed the death time of the others. This protection was not seen if PRVFV was given 24 hours prior to inoculation with NYFV. Similarly, it was shown that NYFV protects against pantropic yellow fever virus (PYFV) but PYFV did not protect against NYFV.

d. Incomplete Virus

Isaacs[92] preferred limited use of the term incomplete virus and restricted its application to focusing attention on the differences in the infectivity of virus produced from serial passage of low-dilution and high-dilution material. He criticized use of the term to imply the production of an abnormal end-product of virus multiplication or the production of developmentally incomplete virus. Hirst[93] ascribed the low infectivity of material produced by serial passage of large inocula of PRVFV to the production of incomplete noninfective virus. He found that mouse serum with a PRVFV titer of 1×10^3 MIVLD$_{50}$ per 0.03 ml, passaged undiluted in mice, dropped as low as $1 \times 10^{1.9}$ MIVLD$_{50}$ per 0.1 ml at the fifth passage. The hemagglutinin titer of this material was not reduced in proportion to the infectivity titer, but remained relatively constant after the first passage. Material diluted to 10^{-3} produced high infectivity titers and proportionately high hemagglutinin titers.

D. STABILITY

1. Acid Tolerance

Findlay[22] reported that PRVFV in blood adjusted to pH 6.9 to 7.3 with phosphate buffer retained viability for 20 hours at 37°C but was

inactivated at room temperature. At pH 6.6 at room temperature, some virus remained viable; however, at pH 8 the virus was inactivated both at room temperature and at 37°C. In contrast, Kaschula[17] reported that maximum viability of the virus was sustained at approximately pH 8. Mims[36] tested virus stability at various pH values from 6.0 to 3.0 at 37.5°C in Sorensen buffer, Sorensen-buffered saline, bovine albumin, and urea concentrations of 0.3 and 3.0 per cent. He found that the virus was equally stable in all the diluents tested as long as the pH was between 7 and 8. Below pH 6.0 the virus was rapidly inactivated. Mims concluded that the absence of virus from the urine of infected mice could probably be attributed to the pH of mouse urine, normally between pH 4 and 6 and rarely as high as pH 7.

2. Heat

PRVFV in phosphate-buffered blood (pH 7.2) retained virulence for 20 minutes at 56°C but not for 40 minutes, according to Findlay.[22] Mims[34] found that undiluted mouse serum with a PRVFV titer of 1 x 10^9 MICLD$_{50}$ per 0.03 ml lost only one to two logs of titer when heated to 56°C for one hour. Surprisingly, the titer did not drop below 1 x 10^5 MICLD$_{50}$ per 0.03 ml when the sample was heated to 56°C for three hours. In contrast, the hemagglutinin of PRVFV was completely destroyed in one hour at 56°C.

3. Chemicals

Daubney et al.[1] recovered viable PRVFV from blood plasma after salting out virus and protein fractions with ammonium sulphate buffered at pH 7.4. Andrewes and Horstmann[33] treated PRVFV with ethyl ether for 18 to 24 hours at 4°C. They added ether to a mouse liver suspension with a titer of 1 x 10^4 MIPLD$_{50}$ per ml and found that the titer was reduced 100-fold. Mims[34] found that RVFV extracted from infective mouse serum with acetone and ethyl ether lost its infectivity but retained antigenicity and hemagglutinin.

4. Storage

a. Cold

According to Daubney et al.,[1] PRVFV in citrated blood may be maintained for one week at room temperature without loss of infectivity. With oxalate-carbol-glycerine (O.C.G.) added, infective blood stored under refrigeration at 5°C (with considerable variation) retained virulence for 54 days and remained viable with reduced virulence for 147 days. Findlay[22] reported that infective blood with O.C.G. remained viable for eight months under refrigeration at 4°C, but with reduced virulence. Defibrinated blood with 0.5 per cent phenol remained virulent for six months at 4°C. PRVFV in undiluted mouse serum remained viable for eight months and showed relatively no loss in titer for 30 days at -20°C.[36] Lunyo virus, stored under the same stated conditions, lost 1.2 logs of virus in 28 days.[32]

Kaschula[17] confirmed Endo's finding[96] that RVFV was most stable in whole egg. This stabilizing action was later attributed to egg yolk. Kaschula concluded that the virus was most stable in undiluted egg embryo at -70°C. Instability of RVFV in mouse brain suspension[97] was attributed to the action of virus-inactivating enzymes, the activity of which was retarded by 0.01 M KCN, condensation products of gallic acid and formalin, and phosphorylation products of resorcinal.[3]

b. Lyophilization

PRVFV in mouse blood dried *in vacuo* over P_2O_5 and maintained at 4°C remained active for at least six weeks.[22] Dried from the frozen state *in vacuo* over H_2SO_4 and maintained at 4°C, the virus in blood diluted 1:10 with normal serum remained viable for eight months.[38] Lyophilized NRVFV in mouse brain lost titer when stored at 37°C for one week or at 4°C for three months. It was found that the presence of buffering electrolytes was harmful to the stability of lyophilized preparations. Stability was improved by the addition of five per cent sucrose, one per cent mixture of 21 amino acids, and five per cent peptone or a saturated solution of lactalbumen hydrolyzate.[3] A lyophilized preparation of Lunyo virus in mouse brain with 100 per cent normal serum as the desiccating medium dropped from a titer of $1 \times 10^{6.8}$ $MICLD_{50}$ to a titer of $1 \times 10^{4.5}$ $MICLD_{50}$ after storage for six weeks. A similar preparation employing beef-peptone-albumin as the desiccating medium lost 4.2 logs of potency after 18 days' storage at -20°C.

E. IDENTIFICATION PROCEDURE

1. Isolation

Rift Valley fever virus has been successfully isolated by injection of suspected material into highly susceptible animals such as lambs or sheep,[1] mice,[72] and ferrets.[27] Isolation has also been made by inoculation of infected material into the yolk-sac or on the chorioallantoic membrane of eight-day embryonated eggs.[17] Whole blood,[1] serum,[22] throat and nasal washings,[27] homogenates of insects,[5] egg embryo,[17] and vital organs such as brain, liver and spleen from suspected sources have been successfully used as inocula.[22] Virus has been concentrated from suspension by precipitation and filtration.[1] Whole blood and serum, aseptically drawn from disease-free animals, have been widely used to obtain bacteria-free suspensions of virus. The method requires knowledge of when and where virus is found in various animals. It has been shown[98] that the titer of pantropic virus in various organs was related to concentration of blood in the organs. The periods during which the more important host species have been found to have virus in the blood are compiled in Table VI.

TABLE VI. CIRCULATION OF PRVFV IN BLOOD OF VARIOUS HOSTS

HOST	DAYS OF PEAK TITER	DAYS AFTER INOCULATION VIRUS PERSISTS IN BLOOD	LITERATURE CITED
Sheep	3-5	6-7	22
Monkey	4-6	13	22
Rat	3-5	15	22
Human	1-3	9	22
Mice	1-2	2-4	36
Lambs	2-3	2-3	36
Ferrets	2-4	2-4	27

2. Identification

a. Cross-Immunity

Thus far, positive identification of Rift Valley fever virus has been accomplished by the adaptation of accepted serological techniques. The earliest test used for the identification of the virus was introduced by Daubney et al.,[1] who performed cross-immunity tests on lambs. Seasonal availability, manipulation, and expense incurred in the extensive use of lambs for routine work were serious limitations of this method. Findlay[99,100] found no cross-immunity between Rift Valley fever and louping ill or dengue in monkeys.

b. Serum Neutralization

Findlay[22] discovered the high susceptibility of mice. He inoculated mice with dilutions of infected blood neutralized with a constant amount of antiserum. Modifications of this test have been applied to differentiation of neurotropic and pantropic strains by intradermal, intracerebral, subcutaneous, and intraperitoneal inoculation.[34] Findlay[21] also showed that the neutralized virus could be reactivated by simple dilution or nasal instillation. Inactivation of nonspecific inhibitors by heating at 56°C for 30 minutes has been recommended.[17]

c. Complement Fixation

The complement-fixation test was adapted to the identification of Rift Valley fever virus and its homologous antisera by Broom and Findlay in 1932.[101] They showed that fixation was in direct proportion to severity

of clinical disease. Exact correlation between the serum-neutralization test and the complement-fixation test was reported by Gear et al.[12,13] It was reported that complement-fixing antibodies were not always detectable in the sera of humans and animals inoculated with the neurotropic strain, but the report was not supported by experimental evidence. Iwasa[84] studied the production of complement-fixing antigen in tissue culture (CHL cells), using the fluorescent antibody technique.

d. Hemagglutination Inhibition

Mims and Mason[94] adapted the hemagglutination inhibition test (HAI) to the identification of Rift Valley fever virus. They showed a specificity of the test for the virus and no serological overlap with Semliki forest (Group A) or yellow fever (Group B) viruses. Optimal hemaggultination occurred at pH 6.5 and 25°C. Acetone-ether-treated and untreated virus were adsorbed to red cells. The virus was not eluted, nor could it be washed off. The untreated virus preparation was a more powerful hemagglutinin than the acetone-ether-treated preparation, which was noninfective. Chick red blood cells were routinely used for the test.

e. Agar Diffusion

Although substantiating evidence was not presented, Weiss[3] reported demonstration of a precipitin reaction with neurotropic virus and its antibody in agar plates.

F. CLASSIFICATION

Casals[102] grouped arthropod-borne viruses into three groups (A, B, and C) on the basis of serological interactions. A fourth group (unclassified) listed viruses that were not placed in the A, B, or C groups because they failed to show serologic relationship to any of the other arthropod-borne viruses. Rift Valley fever virus was placed in the unclassified group. Polson and Linder[74,75] grouped eight neurotropic viruses into three groups on the basis of ultracentrifuge sedimentation rates of 70, 170, and 450 Svedbergs. Rift Valley fever virus fell into the 450-Svedberg group, along with neurotropic horse-sickness virus. Bauer and Bradley[103] classified neurotropic viruses according to xanthine oxidase activity in infected mouse brain. They placed neurotropic Rift Valley fever virus in group two of their five groups. Bunyamwera, encephalomyelitis, neurovaccinia, pseudolymphocytic choriomengitis, Pantz and California viruses were also placed in this group.

IV. SUMMARY

Daubney et al.[1] and Weise[3] suggested that RVF was present in Central Africa for years before 1930. Montgomery (1912) and Stordy (1913) described a similar disease in the Rift Valley, but in neither instance was the etiological agent determined.

Findlay and co-workers described the pathology and histopathology of the disease and adapted the serum-neutralization test and the complement-fixation test to detection of RVFV antibodies in serum in 1932.[22] In 1934 Kitchen made a detailed report on the disease in man.[38] In 1936 MacKenzie and Findlay developed a neurotropic strain (NRVFV) of the virus by serial passage in mouse brain.[72] In the same year the complement-fixation test was used to outline the geographical area of the disease in Africa by extensive testing of sera from African natives.[6]

Smithburn et al.[5] established arthropod transmission of the virus in 1948 by isolating it from mosquitoes caught in the uninhabited Semliki forest in Western Uganda. Subsequently, in 1949, they succeeded in passaging the virus in experimental animals by mosquito bite.[28] In 1950-1951 the first known epizootic of the disease occurred in South Africa.[104]

Takemori et al.[81] reported plaque formation by RVFV in monolayer tissue culture in 1955. In 1956, Weinbren et al.[22] isolated the Lunyo virus, which produces cannibalism in mice. RVF epizootics reappeared in local areas of Africa in 1953, 1955, and 1956.[3] At this writing, the disease has not been reported under natural circumstances outside Africa. Laboratory infections have been reported in Africa, England, Japan, and the United States.[2]

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